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Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation

Ana M. Puga[a], Ana Catarina Lima[b,c], Joao F. Mano[b,c], Angel Concheiro[a], Carmen Alvarez-Lorenzo[a,*]

[a] Departamento de Farmacia y Tecnología Farmacéutica, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
[c] ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

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ABSTRACT

5-Fluorouracil (5-FU)-loaded chitosan microgels for oral and topical chemotherapy were prepared applying a superhydrophobic surface-based encapsulation technology. Drug-loaded chitosan dispersions were cross-linked and then coated with drug-free chitosan or pectin layers at the solid-air interface in a highly efficient and environment-friendly way. The size of the microgels (with diameters of ca. 280 and 557 μm for the chitosan seeds and pectin-coated microgels respectively) was the lowest obtained until now using similar biomimetic methodologies. The microgels were characterized regarding 5-FU release profiles in vitro in aqueous media covering the pH range of the gastrointestinal tract, and cytotoxicity against two cancer cell lines sensitive to 5-FU. Owing to their control of 5-FU release in acidic medium, calcium pectinate-coated microgels can be considered as suitable for oral administration. Growth inhibition of cancer cells by 5-FU was greater when incorporated to chitosan microgels; these being potentially useful for treatment of skin and colorectal tumors.

1. Introduction

Oral chemotherapy is attracting increasing attention since it can provide convenient home-based therapy with lesser collateral effects than intravenous treatments, leading to a better quality of life (Banna et al., 2010). In fact, more than twenty antitumor drugs have been recently approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for oral administration, and most of the new molecules under research are thought to be developed as oral medicines (Krishnaiah & Khan, 2012; Ruddy, Mayer, & Partridge, 2009). As an example, 5-fluorouracil (5-FU) has been shown suitable for systemic oral therapy, without expecting differences in drug efficacy compared to continuous infusion (Miura et al., 2010). In addition to systemic absorption, the oral route also enables site-specific treatment of colorectal cancer, which has been reported as the fourth most frequent cause of cancer death (Pisani, Bray, & Parkin, 2002). Mucoadhesive formulations can provide high local concentrations of antitumor agents and prolonged contact with the tumor tissue, using lower doses and thus causing less damage in healthy tissues (Haupt, Zioni, Gati, Kleinstern, & Rubinstein, 2006; Krishnaiah & Khan, 2012). Chitosan-coated alginate microspheres (Urbanska, Karagiannis, Guajardo, Langer, & Anderson, 2012) and pellets of hyaluronic acid-coupled chitosan nanoparticles (Jain, Jain, Ganesh, Arve, & Beg, 2010) loaded with oxaliplatin, Eudragit S-100 coated hydroxypropyl methylcellulose granules (Ciftci & Groves, 2006) and gelatin-coated chitosan microspheres (Bhat et al., 2012) loaded with 5-FU, and chitosan microspheres with valdecoxib (Thakral, Ray, & Majumdar, 2012) have been recently shown useful for colorectal cancer treatment. Bioactivation of 5-FU due to the cellular enzymatic activity is notably promoted in colorectal tumor tissue compared to normal tissue (Wei, Qing, De-Ying, Bai, & Li-Fang, 2008). Moreover, chitosan can reduce the side effects caused by anti-metabolite drugs such as 5-FU, particularly the injury to the small intestinal mucosa membrane, the incidence of diarrhea and the reduction of the blood leukocyte number, without loss of antitumor activity (Kimura & Okuda, 1999). Combination of chitosan with 5-FU might be also suitable for the topical treatment of precancerous and cancerous lesions of the skin (targeting to atypical keratinocytes) and tumors affecting vaginal-uterine tissues (Lam et al., 2012; McGillis & Fein, 2003). Chitosan may increase the penetration of 5-FU through the epithelial cells and, thus, enhance the effectiveness of the treatments (Sabitha et al., 2013).

The design of microparticles for drug delivery has to face up to two relevant difficulties: (i) small and hardly predictable drug entrapment efficiency and (ii) poor control of the drug release

* Corresponding author. Tel.: +34 981563100x15239; fax: +34 981547148.
E-mail address: carmen.alvarez.lorenzo@usc.es (C. Alvarez-Lorenzo).

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rate through the short diffusion pathway (Bhattarai, Gunn, & Zhang, 2010; Sinha et al., 2004). Recently, a bioinspired approach to obtain polymer microgels with 100% encapsulation yield has been developed exploiting the way aqueous droplets adopt spherical forms on superhydrophobic surfaces, followed by a step of hardening/cross-linking of the polymer at the solid-air interface (Lima, Song, Blanco-Fernandez, Alvarez-Lorenzo, & Mano, 2011; Song, Lima, & Mano, 2010). This methodology avoids harsh conditions and organic solvents and may enable a very precise and reproducible dosing in the microgels of narrow therapeutic range, labile drugs and molecularly targeted proteins and peptides used in the cancer treatment.

The aim of this work was to develop chitosan-based microgels for oral administration or skin/mucosal application of 5-FU, implementing a methodology consisting of (i) deposition of droplets of drug-containing chitosan aqueous solutions on superhydrophobic surfaces, (ii) cross-linking of chitosan in glutaraldehyde atmosphere, and (iii) optionally, coating of the microgels by successive deposition and cross-linking of drug-free chitosan or other polysaccharide (e.g. pectin) solutions in order to attain a better control of drug release. Former trials with bioinspired superhydrophobic surfaces involved acrylic-modified polymers and UV polymerization (Lima, Custodio, Alvarez-Lorenzo, & Mano, 2013; Lima et al., 2011). As an advantage, the present work avoids monomeric species and initiators and employs natural polysaccharides (Muzzarelli et al., 2012), being a safer and greener approach. To carry out the work, first superhydrophobic surfaces that mimic the hierarchical micro- and nano-structure and the roughness of the Lotus leaves were obtained on polystyrene in order to attain water contact angles greater than 150° and sliding angle lower than 10° (Neto, Custodio, Song, & Mano, 2011; Song et al., 2010). In addition to chitosan solely microgels, semi-interpenetrating networks (semi-IPN) of chitosan with hyaluronic acid and different varieties of Eudragit® were prepared. Sodium hyaluronate (SH) has been shown able to modulate cell adhesion, growth and migration, wound healing and cancer metastasis (Jain & Jain, 2008; Zeng, Toole, Kinney, Kuo, & Stamenkovic, 1998). Moreover, hyaluronic acid receptors such as CD44 and Rhamm are overexpressed in cancer cells, including colorectal ones (Kobel et al., 2004). Two acrylic copolymers namely Eudragit® E PO (soluble at pH > 5) and NE 30 D (time-dependent dissolution) were included in the microgels to explore the possibilities of tuning 5-FU release rate as a function of pH (Lunter & Daniels, 2012; Paharia et al., 2007). After hardening, some chitosan microgels were coated, also on the superhydrophobic surfaces, with pectin cross-linked with $CaCl_2$ in order to facilitate colon specific release (Liu, Fishman, Kost, & Hicks, 2003; Sriamornsak, 2011). The developed approach may enable a precise regulation of the amount of drug encapsulated and the pectin-coated microgels provide pH-responsive controlled drug release.

2. Materials and methods

2.1. Materials

Polystyrene sheets (60 mm × 60 mm) from square Petri dishes (Bdbioscience, Enzifarma, Portugal) and polystyrene granules of injection molding grade were used for the obtaining of syperhydrophobic surfaces (Song et al., 2010). Chitosan (chitosan 222, average deacetylation degree 76.2%, Mw 359,000 (s.d. 11,570) Da (Barreiro, Coronilla, Concheiro, & Alvarez-Lorenzo, 2005)) was from George S. Daras (Marseille, France); sodium hyaluronate (SH) was from Guinama (Valencia, Spain); and 5-fluorouracil (5-FU) was from Fagron Iberica S.A.U. (Barcelona, Spain). 1H,1H,2H,2H-Perfluorodecyltriethoxysilane (PFDTS, 97%), crystal violet, formic acid, fetal bovine serum (FBS), glutamine, non-essential amino acids (NEAA), pectin from apple (galacturonic acid content 77.5%, methoxy content 7.8%), pectin from citrus fruits (galacturonic acid content 84%, methoxy content 10%) and penicillin–streptomycin solution were from Sigma–Aldrich Co. (St. Louis, MO, USA). Dulbecco' Modified Eagle's Medium (DMEM) with high glucose content was from Gibco (Invitrogen, Spain) and Eagle's Minimum Essential Medium (EMEM) from the American Type Culture Collection (Manassas, VA). Glutaraldehyde solution (25%, v/v), calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) and orthophosphoric acid (85%) were from Merck (Hohenbrunn, Germany). Eudragit® E PO and NE 30 D were provided by Evonik (Germany) and 2-(4-morpholine)ethanesulfonic acid (MES) by Fischer Bioreagents (Leicestershire, UK). Purified water (resistivity > 18 MΩ cm; MilliQ®, Millipore, Spain) was obtained by reverse osmosis. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation and characterization of polystyrene superhydrophobic surfaces

Polystyrene sheets were washed with ethanol in an ultrasonic bath for 15 min and treated with a solution of polystyrene (70 mg/ml) in tetrahydrofuran and ethanol (2:1.3, v/v). Then, the sheets were immersed in ethanol for 1 min and dried under nitrogen flow before argon plasma treatment (30W; Plasma Prep5, Gala Instruments, Germany) for 20 s. Finally, the sheets were immersed in a PFDTS solution (1% in ethanol) for at least 24 h, and then removed and air dried. The roughness of the surfaces before and after treatment and after being used were observed by means of Scanning Electron Microscopy (SEM, FESEM ULTRA Plus instrument, Zeiss, Oberkochen, Germany). Water contact angle was measured at room temperature in static mode using a Phoenix 300 goniometer (SEO, Korea).

2.2.2. Purification of chitosan and Eudragit® E PO

Chitosan was purified following a precipitation procedure previously described (Signini & Campana Filho, 1999). Briefly, chitosan (10 g) was dispersed in acetic acid 2% (v/v) and filtered first through a cellulose membrane of 7–11 μm pore size (ALBET-Hahnemühle, Barcelona, Spain) and then through a nylon membrane of 0.45 μm pore size (Lida Manufacturing Corp., Kenosha, WI, US). The pH of the solution was increased up to 8 with NaOH 2 M and the precipitated chitosan was washed with water until the medium reached pH 7, and then with ethanol:water 80:20 and 90:10 (v/v). The purified chitosan was freeze-dried. Purification of Eudragit® E PO was carried out by dialysis of a solution of the acrylic polymer (5 g) in phosphate buffer pH 2 (50 ml) against phosphate buffer pH 2 for 2 days, and against water for 5 days more using dialysis tubes of 12,400 Da MWCO (Sigma–Aldrich Co., St. Louis, MO, USA). Finally, purified Eudragit® E PO was freeze-dried.

2.2.3. Preparation of chitosan microgels

Dispersions of chitosan, chitosan/SH, and chitosan/Eudragit® in acetic acid 1% (v/v) were prepared at various concentrations (Table 1). 5-FU was added to aliquots of the solutions up to 0.5% (w/v). Droplets (2.5 μl) of the polymer/s solutions containing 5-FU were placed on the superhydrophobic surface of polystyrene sheets using a micropipette. Then, the sheets with the droplets were transferred to a dessicator containing 500 ml of glutaraldehyde solution (25%, v/v in water) at the bottom in order to create a glutaraldehyde atmosphere able to cross-link the microgels. After 1 or 4 h, the microgels were transferred to another dessicator that was connected to vacuum for 4 h for removal of unreacted glutaraldehyde. Chitosan cross-linking was monitored by FTIR analysis (Bruker model IFS-66v, Bruker Optics, Karlsruhe, Germany) of the